

## EFFECT OF NATURAL FERMENTATION ON THE NUTRITIONAL QUALITY OF "EL HAMMOUM" DURUM WHEAT (*TRITICUM DURUM*) FERMENTED PRODUCT OF THE ALGERIAN COUNTRY

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### ABSTRACT

In Algeria, El-Hammoum, durum wheat fermented, from storage in underground silos (matmoras) in some rural environment, is appreciated for its organoleptic properties and its richness in protein, good for health; it is used as a remedy by diabetics, because of its poverty of sugars linked to its fermentation. The aim of the study is the characterization of the El-Hammoum biochemical composition and that of profile its main metabolites which have health effect. Compared to unfermented wheat, El-Hammoum was rich in water, protein and total polyphenols representing increases of 3.46%, 4.9% and 29.6% and low content of pH, PMG PS, total sugars, starch, amylopectin and gliadin respectively reductions of 10.35%, 17.3%, 23.4%, 40.4%, 34.2% 47 % and 57.3%. The richness of polyphenols (antioxidants) and protein of fermented wheat confers him a beneficial effect on health. The Low contents of total sugars and starch would justify its use by the local population diabetics. The decrease in gliadin, gluten protein fraction, implicated in celiac disease, would be beneficial for these patients.

**KEYWORDS:** Durum Wheat, El-Hammoum, Fermentation, Matmora, Diabetics, Physicochemical, Phytochemical

### INTRODUCTION

In rural environment, the traditional use of some foods or medicinal plants to treat diseases is still used today. In Algeria, an ethnopharmacological survey showed a particularly interesting use of El-Hammoum by diabetics. El-Hammoum results from the fermentation of durum wheat (*Triticum durum*) known by the Arabic and Berber vernacular names "Guemah" and "Timzin" respectively; it is the most cultivated cereal.

In the past, farmers conserved wheat grains during several years by using individual or collective underground pits: generally with truncated form which becomes wide at the bottom, known by the vernacular name "Matmora": a traditional practice of storing grains in an adequate confined atmosphere. Initially, inexperienced farmers didn't master the choice of soil for digging their own pit, which involved a spontaneous fermentation of the grains in contact with Matmora's walls, in low drained soils (Cruz et al., 2002). The obtained fermented wheat, considered as a loss by botanists, is exploited by the farmers and known by vernacular name "El-Hammoum" which comes from "Hmoum" meaning black, referring to the black colour of fermented wheat.

El-Hammoum, at first, was appreciated for its organoleptic properties and became a local product, especially in rural areas where it is consumed in the form of couscous, an Algerian food.

Several authors reported that the spontaneous fermentation of starch products, such as cereals, is of lactic or acetic acid type (**Viéra-Dalodé et al., 2007**).

Many studies highlighted the association of a regular consumption of fermented products, resulting from lactic fermentation, with a reduction in the risk to develop certain diseases such as diabetes and cardiovascular diseases (**Guyot, 2010**). The compounds involved in these effects are found in protein fractions, fibers and especially in molecules coming from the plant and Lactic acid bacteria secondary metabolism, including vitamins, triterpens or polyphenols that can act alone or in interaction (**Fardet et al., 2008**).

In order to highlight the features of El Hammoum, where currently no study was devoted to the characterization of this product; the prime objective of this work consisted in the characterization of its biochemical composition and that of its main metabolites profile for health purpose. The second objective was to determine the variability of some group of molecules contained in El-Hammoum, targeted according to their biological interest.

## **MATERIALS AND METHODS**

This study was conducted in the laboratories of the Faculty of nature and life sciences, Ibn Khaldoun University, Tiaret-Algeria.

### **Plant Material**

The durum wheat variety (VITRON), used in this study, was obtained from July 2006 harvest and was stored until May 2011 in a Matmora located in Tiaret (southwestern of Algeria, altitude 1040 m); hermetically sealed at the opening with clay in order to isolate the content from outside air and pests, and whose walls are lined with wheat straw. The fermented wheat (FW) was retrieved from the walls, unfermented wheat (UFW), in the center, served as a control sample.

### **Methods**

Wheat samples (FW and UFW) were cleaned with water and then dried at the ambient air (technique used by farmers before wheat grinding). The analysis focused on the whole grains or their grinding.

### **Physicochemical Analysis**

Test weight (TW), determined using the Nilema-litre (TRIPETTE RENAUD) according to standard **NF V 03-719**, and 1000 kernel weight (TKW) were performed on whole grains for all samples according to standard **NF V 03-702**. On the milling of each type of whole wheat, were determined: The moisture content (%) **ISO 712**, total ash (%) **ISO 2171**, fall time Hagberg: **NF ISO 3093** and pH measurement was performed using a pH-meter Karl Kolb.

### **Biochemical Analysis**

The protein content by the kjeldahl method (**AFNOR V03-607**). The extraction of different classes of proteins, based on their solubility, was made according to the protocol described by (**Osborne, 1924**), wet gluten (%) **ISO 21415-2** and dry gluten (%) **ISO 21415-4**. The extraction of different classes of proteins, based on their solubility, was made according to the protocol described by **Osborne (Osborne, 1924)**, the protein concentration (mg / g dry weight) of each fraction was determined by the method of Bradford (**Bradford, 1976**) in reference to a standard of BSA (bovine serum albumin) and the optical density was read at 595 nm.

The total sugars (g/100g) were determined by the method of Dubois (**Dubois et al., 1956**) using glucose as standard, the absorption of the color produced was read at 490 nm. The starch content was affected by the polarimetric method of Ewers (1965), amylopectin and amylose were determined by fractionation of starch in 1-butanol / water (1:7) (**Cura and Krisman, 1990**) and insoluble fiber by the method Weende (%) **NF V 03-040**.

### Phytochemical Screening

For quantification of total polyphenols, we proceeded to a methanol extraction (90v/10v) (**Zielinski and Kozłowska, 2000**). Their content was determined by the Folin-Ciocalteu method of Singleton and Rossi (**Velioglu et al., 1998**) using gallic acid as standard.

The absorption of the color produced is read at 750 nm, the results are expressed in mg GAE/100g dry weight of plant material.

Phytochemical Screening and characterization of substances were made on extracts obtained by successive maceration by three solvents polarity increasing (diethyl ether, dichloromethane and methanol) was used at room temperature (**Diallo et al., 2004**). Tannins detection test by FeCl<sub>3</sub> (**Karumi et al., 2004**). Anthocyanins by acidification H<sub>2</sub>SO<sub>4</sub> to (10%) followed by alkalization with NH<sub>4</sub>OH (10%) (**Bruneton, 1999**), Anthraquinones by adding NH<sub>4</sub>OH (10%) (**Oloyede, 2005**) and saponins (**Koffi et al., 2009**).

### Microbiological Analysis

The samples were taken in a sterile way in matmora. The Search for yeasts and molds was made on the OGA environment, at 25 ° C for 3 to 5 days (**Guiraud, 2003**). Isolation of lactic acid bacteria was done on MRS and M17 media at 42°C for 24-48 h. The isolated strains were tested according to their phenotypic characteristics morphology, Gram reaction, catalase and oxidase activity.

### Statistical Analysis

Results presented are the mean values  $\pm$  standard deviation (SD) of three independent assays. Data were compared by Student test. The statistical significances ( $P < 0.05$  and  $P < 0.001$ ) were determined by using STATISTICA (V. 6.1) software.

## RESULTS AND DISCUSSIONS

### Morphological Aspect of Fermented Wheat

The very pronounced mold smell that came from FW samples can be explained by the production of volatile and aromatic compounds during fermentation.



Figure 1: Picture of Fermented and Unfermented Wheat. UFW: Unfermented Wheat FW: Fermented Wheat

The brown color observed (figure 1) can be explained by the non-enzymatic browning, as has been observed in other studies on food fermentation (Faithong and Benjakul, 2012). A particular taste acid was also perceived.

### Physicochemical Characteristics of the Grains

Results of the physicochemical analysis are shown in Table 1. Statistical analysis shows a significant difference between FW and UFW.

**Table 1: Physicochemical Characteristics of the FW and UFW**

<i>Unfermented Wheat Average <math>\pm</math> SD</i>		<b>Fermented Wheat Average <math>\pm</math> SD</b>
<i>TKW (g)</i>		$44 \pm 1.55^{**}$
<i>TW (kg/hl)</i>	$82.45 \pm 2.19$	$63.15 \pm 0.21^{**}$
<i>Ash (% DW)</i>	$1.714 \pm 0.025$	$1.729 \pm 0.026$
<i>Moisture content (%)</i>	$11.84 \pm 0.05$	$12.25 \pm 0.06^{***}$
<i>pH</i>	$6.28 \pm 0.021$	$5.63 \pm 0.014^{***}$
<i>FN (Seconds)</i>	$384 \pm 16.64$	$64 \pm 2.65^{***}$

*P<0.0001 Very Highly Significant Difference, p<0.001 Highly Significant Difference*

### Test Kernel Weight and Test Weight

The TKW and TW of FW have undergone reductions of 17.3% and of 23.4% ( $p<0.05$ ) respectively. This decrease is due partly to the loss of dry matter and on the other hand the increase of the water content (Dexter and Edwards, 1998).

### Ash Content

No significant difference between FW and UFW has been observed, fermentation did not affect this parameter.

### Moisture Content

An increase of 3.46 % ( $p<0.001$ ) in moisture content was seen in FW (Table 1 and figure 2). According to Bartali (Bartali *et al.*, 1989), fermented wheat that is near the walls of the Matmora has high water content.

### The pH and Wheat Falling Number

The fermented wheat is more acidic than the unfermented wheat, its pH is lower. The wheat falling number is very low compared to that of unfermented wheat with a very highly significant difference ( $P<0.001$ ).

### Biochemical Composition

The results of the biochemical analysis are shown in Table 2.

### Proteins

The protein fractionation of two types of wheat was very interesting regarding the variation of the different protein fractions. It revealed: a hydrolysis of glutenin and gliadin fractions in FW compared to UFW that is a decrease of 20% and 57.3% respectively (figure 2). This may partly explain the depolymerization of gluten which is the cause of the non-formation of a paste during gluten content determination.

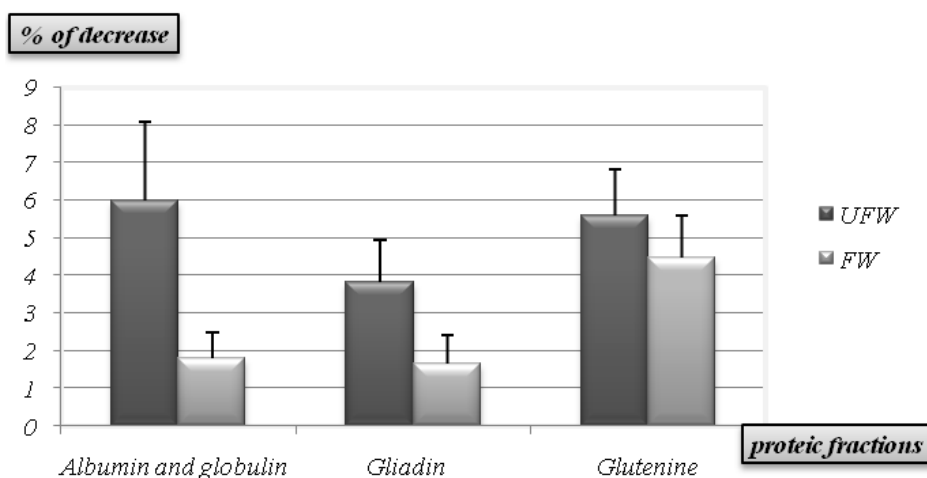
Similar findings have been reported in other studies, but with a greater rate of hydrolysis than that found in our sample, this being dependent on the bacterial strains involved (**Thiele et al., 2004**): a strong hydrolysis of albumins at a rate of 83.4% and a smaller decrease 16.8% of globulins.

However, the elevation of the protein content 4.9% ( $p < 0.001$ ) in the FW (Table 3 and Figure 3) can be explained by bacterial synthesis of new protein products such as amino acids, bacteriocins,... (**Kohajdova and Karovicova, 2007**).

### Carbohydrates

The fermented wheat has a low falling number, which means a very high amylase activity. However, excessive presence of  $\alpha$ -amylase causes a decrease ( $p < 0.05$ ) of starch content that went from 73.34% in the UFW to 48.17% in the FW (that is a decrease of 34.2%), therefore a decrease in total sugar content of 40.4%. This decrease can be explained by the production of organic acids as a result of lactic acid bacteria activity (**World Health Organization**) which resulted in the decrease of the pH (**Kohajdova and Karovicova, 2007**) at a rate of 10.35% observed in the Hammoum.

The modification of starch in the FW, that would be linked to a significant ( $p < 0.05$ ) degradation of amylopectin of  $47.45 \pm 0.93\%$  (UFW) to  $22.36 \pm 1.01\%$  (FW), amylose content was slightly affected of 25.89 (UFW) vs. 25.7% (FW). The amylopectin is the dominant fraction and more assimilable (**Bornet, 1993**); results in a low glycemic load after Hammoum consumption, hence the interest of diabetics for this local product. In addition, high amylase activity associated with the degradation of gluten affects the rheological characteristics of pasta, they have as a consequence the deterioration of breadmaking quality, and this explains the Hammoum consumption only in the form of couscous.



**Figure 2: Decrease of Proteic Fractions Expressed in %**

The characteristics of unfermented wheat are showed in Table1 and 2. The content of the various parameters studied are within the limits of the values published by **Godon (Godon, 1991)**: water (12-16%), carbohydrates (starch and sugars: 63 to 74.5 %), proteins (10-15%), cellulose (2.5 to 6%), minerals (1.8 to 2.2%); only the gluten content obtained seemed to be affected (**Guinet, 1992**); this may be due to the long-term storage of wheat in the Matmora.

**Table 2: The Biochemical Composition of the FW and UFW**

UFW	FW
Average $\pm$ SD	Average $\pm$ SD
Proteins (% DW)	15.33 $\pm$ 0.03
	16.08 $\pm$ 0.014***
Total sugars (g/100g)	81.08 $\pm$ 1.12
	48.67 $\pm$ 0.05**

Table 2 : Contd.,		
Cellulose (% DW)	4.135 ± 0.23	4.175 ± 0.09
Starch (%)	73.34 ± 1.55	48.17 ± 3.09**
Amylopectin (%)	47.00 ± 0.93	22.36 ±
Wet gluten	18.50 ± 1.51	-
Dry gluten	6.8 ± 0.66	-

\*\*\*P<0.0001 Very Highly Significant Difference. \*\*p<0.001 Highly Significant Difference

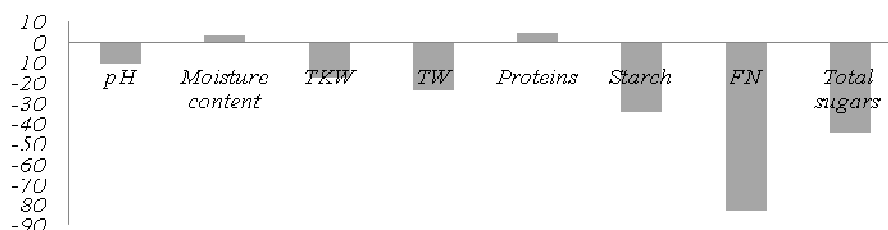


Figure 3: Variation of Different Parameters Expressed in %

### Phytochemical Screening

The results showed a significant increase in the total polyphenol content in the fermented wheat compared to unfermented wheat, that is an increase of 29.6% (Table 3); which is not in perfect agreement with some studies (El Hag et al., 2002) reporting that the total polyphenols decrease during fermentation; other works done by (Jung et al., 2010) on lactic acid fermentation showed that the levels of polyphenols increase. This increase in polyphenol content during fermentation is possible only with certain *Lactobacillus* strains (Eom et al., 2011) on the one hand and on the other hand, it is due to the presence of certain types of phenolic compounds, such as isoflavones, particularly the aglycone subgroup, which increase significantly during the lactic acid fermentation (Hubert et al., 2008).

This explains our results because the fermented wheat underwent lactic fermentation firstly and secondly, wheat is known to contain aglycones in its polyphenol profile (McCallum, 1989). Moreover, the richness of Hammoum in polyphenols gives it positive effects on health; protection against cardiovascular disease, type II diabetes, obesity, anticarcinogenic action, fight against cellular aging (Serpen et al., 2008).

For other chemical groups studied, only the test of saponins is very positive for the fermented wheat compared to unfermented wheat which is moderately positive. The detection reactions for tannins, anthocyanins, anthraquinones and alkaloids were negative; this would be due on the one hand, to the absence of adequate methods and not adapted to cereals; secondly, the low concentration of these compounds in wheat compared to other plants in general, and other cereals in particular (McCallum, 1989).

Table 3: Total Polyphenols Content

UFW	FW
Average ± SD	Average ± SD
polyphénols totaux (mg EAG/g)	18,32 ± 0,37
	23,75 ± 2,9*

\*p<0.005 Significant Difference

### Microbiological Characterization of "El Hammoum"

Lactic acid bacteria were found in the fermented wheat and absence in the UFW, This suggests that the fermentation in the "matmora" was type lactic. The Examination of lactic acid bacteria showed the coexistence of two families: the *Lactobacillaceae* and *Streptococcaceae*.



UFW-OGA

FW-OGA

**Figure 4: Isolation of Molds on the Middle OGA**

The Yeasts are present in small numbers in the two samples, but much lower in the fermented wheat figure 4. The presence of mold is low in the non-fermented wheat, appears absent in the fermented wheat, this can be justified by storage conditions in confined atmosphere that do not favor fungal development and secondly, the lactic acid bacteria are known to produce various antimicrobial compounds which are inhibitory to bacteria and fungi (Hassan and Bullerman, 2008).

Moreover other studies have shown those substances such as acid phenyllactic and 4-hydroxy-phenyllactic acid and a range organic acids acetic acid, formic acid, caproic acid, propionic, butyric and valeric released by *Lactobacillus* showed inhibitory activity against fungi (Gobbetti *et al.*, 2005).

## CONCLUSIONS AND PERSPECTIVES

The significant variations found between non-fermented wheat and El-Hammoum for the different components studied assert the influence of the fermentation by improving the nutritional value of wheat through an increase in proteins and the biological value, that is increase in polyphenols, notable decreases in starch, total sugars and gliadin fractions. El-Hammoum would be a very interesting perspective on the diet for diabetics and those predisposed to celiac disease. From which an interest to continue this work on the study of the fermentation process in order to better control fermentation. Moreover, further detailed studies on El-Hammoum are needed to better understand the characteristics of El-Hammoum biologically.

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